MC1R, ASIP, and DNA Repair in Sporadic and Familial Melanoma in a Mediterranean Population

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Background: **Melanoma risk factors include fair pigmentation, multiple nevi, low DNA repair capacity, and CDKN2A or CDK4 mutations. Variants of the melanocortin-1 receptor (MC1R) gene have been associated with fair pigmentation and melanoma risk, and a polymorphism of the Agouti Signaling Protein (ASIP) gene has been associated with dark pigmentation. We examined MC1R and ASIP genotypes in relation to phenotypic characteristics, sporadic and familial melanoma risk, and melanoma thickness as an indicator of disease progression in a Mediterranean population.** ***Methods:*** **We studied 267 melanoma patients and 382 control subjects from a case–control study and a family study in northeastern Italy. Host factors were assessed by physical examination, questionnaire, spectrophotometer, and minimal erythema dose measurement. MC1R was sequenced, ASIP was genotyped, and DNA repair capacity was measured by the host–cell reactivation assay. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by logistic regression models. Effect modification of the association between MC1R and melanoma risk by phenotypic characteristics and DNA repair capacity was also assessed. All statistical tests were two-sided.** ***Results:*** **Carrying MC1R variant alleles was associated with a two- to fourfold increase in risk of both sporadic and familial melanoma compared with carrying wild-type MC1R, particularly in individuals carrying multiple variant alleles (OR = 3.9; 95% CI = 3.3 to 4.6). This association was stronger in individuals with fewer additional risk factors (those with dark skin or few nevi). MC1R variant allele carriers were also three to four times more likely than were non-carriers to have thick melanomas. The ASIP polymorphism was not associated with pigmentation, nevi, or melanoma risk.** ***Conclusions:*** **MC1R was associated with melanoma risk and progression in a Mediterranean population, particularly in the absence of other strong risk factors, such as freckling or many nevi. [J Natl Cancer Inst 2005;97:998–1007]**

A number of host factors have been associated with melanoma risk. Among phenotypic factors, fair pigmentation, low tanning ability, and the presence of many melanocytic nevi or dysplastic nevi (DN) are all associated with increased risk of developing melanoma *(1,2)*. Different risk factors may lead to melanoma formation through different pathways *(3)*. For example, both fair-skinned redheads who are prone to freckling but with few melanocytic nevi *(4,5)* and darker-skinned individuals who have many nevi *(1)* may be at high risk of developing melanoma. Moreover, only 20–30% of melanoma lesions are found to be histologically associated with a preexisting nevus *(6)*, although this figure may be an underestimate because preexisting nevi are hard to detect in advanced melanoma lesions. Additional, nonphenotypic host factors may also play a role in melanoma development. For example, variation in proficiency of repairing ultraviolet (UV) radiation–induced DNA damage is associated with melanoma risk *(7)*. We previously reported that DNA repair capacity modifies the associations between host risk factors, such as low tanning ability or dysplastic nevi, and melanoma *(8)*.

Families at high risk of developing melanoma have provided evidence of additional genetic factors that may be involved in melanoma susceptibility. Studies of large melanoma-prone families have demonstrated linkage of melanoma susceptibility to a locus on chromosome 9p21 (MIM 600160) in the majority of kindreds and probable linkage to 1p22 *(9)* and 1p36 *(10)* in others. About one-third of families in which melanoma risk is linked to 9p21 carry mutations in the CDKN2A tumor suppressor gene *(11)*, which encodes the p16 cell cycle inhibitor. Rare kindreds may also possess mutations in the coding sequences of CDK4 (MIM 12829) *(12)* or $p14^{ARF}$, which is translated from exons 1β and 2 of the CDKN2A gene *(13)*. Another potential candidate gene is CDKN2B, which is also part of the cell-cycle regulation pathway *(51)*.

One low-penetrance gene that has been associated with melanoma risk is the melanocortin-1 receptor gene (MC1R, MIM *155555). Its protein product binds to the α-melanocyte–stimulating hormone (α-MSH) and adrenocorticotropin (ACTH) *(14)*, and regulates pigmentation. MC1R is highly polymorphic, and more than 65 human MC1R alleles with nonsynonymous changes have been identified to date *(15)*. Studies of Northern European populations have identified several MC1R variants that are associated with fair pigmentation phenotypes. In particular, the presence of the Arg151Cys, Arg160Trp, or Asp294His variant of MC1R, any of which is known as a "red hair color" (RHC) variant, has been associated with red hair, low tanning ability, light skin color, and freckling *(16–18)*. Other MC1R variants have a weak or no association with red hair *(18)* and are referred to as "non-red hair color" (NRHC) variants. Several studies in fair-skinned populations of Northern European origin have reported

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that the risk of melanoma is higher among individuals who carry MC1R variant alleles than among individuals who are wild type for MC1R *(17,19,20)*. MC1R can also act as a modifier of melanoma risk within CDKN2A-positive melanoma-prone families *(20,21)*, whereas the risk associated with MC1R variant alleles in CDKN2A-negative families is unknown.

Another pigmentation gene that has the potential to be associated with melanoma risk and is highly conserved across species is the Agouti Signaling Protein gene (ASIP). In mice, its protein product acts as an inverse agonist of the MC1R gene, which may be able to decrease melanogenesis *(21)*. In humans, an A-to-G substitution (g.8818A>G) in the 3′ untranslated region (UTR) of the ASIP gene (MIM, *600201) has been associated with dark hair and eye color among Caucasians *(22)*. However, it is not known whether ASIP is associated with melanoma risk.

We examined the associations of variant alleles of the MC1R and ASIP genes with pigmentation, nevi count, and melanoma risk in a Mediterranean population that is mostly negative for mutations in additional melanoma candidate genes. We also assessed whether these genes and/or DNA repair capacity were involved in disease progression.

Subjects and Methods

Study Population

This study included 267 melanoma patients and 382 control subjects identified from a case–control study and a family study in northeastern Italy. The case–control study, which has been described in detail by Landi et al. *(1)*, included 183 melanoma patients (87 males and 96 females) and 179 control subjects (89 males and 90 females) aged 17–77 years. Melanoma patients had no family history of melanoma. Of the 179 control subjects, 134 were spouses or close friends of the melanoma patients, 14 were outpatients referred to the hospital because of minor accidental trauma, and 31 were healthy volunteers from hospital personnel. Control subjects were frequency-matched to melanoma patients by decade of age and sex. All control subjects were from the same geographic area as the melanoma patients. None of the subjects in this study had mutations in any of the major candidate genes for melanoma, including CDKN2A, CDK4, or $p14^{ARF}$.

The family study, which has been described in detail by Landi et al. *(23)*, included 55 melanoma-prone families, which are defined as having two or more individuals affected with melanoma per family. As in many melanoma-prone kindreds, several individuals with melanoma had dysplastic nevi, multiple melanomas, or, more rarely, pancreatic cancer. Some of the melanoma case patients were deceased at the time of family recruitment; thus, DNA was obtained from 84 melanoma patients and 203 unaffected individuals (23 of whom were spouses, i.e., not blood relatives). All families have been tested for presence of mutations in CDKN2A, $p14^{ARF}$, CDK4, and CDKN2B. Only four families (nine subjects) had mutations in CDKN2A, and no family had a mutation in any of the other genes.

All 649 subjects from both studies were recruited at the Dermatology Department of the Maurizio Bufalini Hospital of Cesena, Italy. During the study period, the Bufalini Hospital examined approximately 85% of all melanoma patients diagnosed in the area, as verified by comparison of patient lists with the Romagna region cancer registry *(24)* and with records of melanoma diagnoses from the main hospitals of the area. Both studies were approved by the Bufalini Hospital and National Cancer Institute's ethical committees, and written informed consent was obtained from all participants. A standardized in-person questionnaire was administered to all participants by trained interviewers, who asked questions on lifetime residential history, medical history, family history of cancer and other diseases, drug consumption (i.e., corticosteroids or photosensitizing antibiotics), skin reaction to the first half-hour of sun exposure, tanning ability after prolonged sun exposure according to the Fitzpatrick score *(25)*, and sunscreen use. Participants in the case–control study were also asked questions about exposure to sun and artificial UV radiation. The entire skin of each participant's body, except for the genital area, was examined by a single dermatologist (DC) to assess pigmentation characteristics, freckling, presence of skin lesions, and presence of other skin cancers.

To assess all diagnoses of dysplastic nevi and verify the number of nevi, an expert oncologist, blinded to melanoma status, was provided with standardized photographs of the back of each subject. Nevus count was restricted to the back to decrease the amount of time spent examining the patient and to maximize subject participation rate. However, restricting nevus count to the back should not negatively impact classification based on this characteristic, since the number of nevi on the trunk has been shown to be highly correlated ($r = .82$ in female Caucasians and $r = .90$ in male Caucasians) with the number of nevi on the entire body *(26)*. Moreover, recognition and classification of nevi and dysplastic nevi from photographs have been shown to be accurate and reproducible *(27)*. To be defined as dysplastic, a nevus had to be 5 mm or larger, be predominantly flat, and have at least two of the following characteristics: variable pigmentation, indistinct borders, and an irregular outline *(8,10,28)*. The study dermatologist assessed the skin, hair, and eye color of each study subject *(1)*.

Standardized Measurements of Skin Color and UV Radiation Sensitivity

In addition to qualitatively examining skin color and sensitivity to the sun, we objectively assessed these traits. Detailed description of the two instrumental assessments has been reported by Brenner et al. *(29)*. Briefly, skin color was measured using a Minolta 300 spectrophotometer/colorimeter (CR-300, Konica-Minolta, Osaka, Japan). The colorimeter measures skin color by measuring reflected light from the visible spectrum (range, 400–700 nm) and records colors in a three-dimensional space of dimensions, L*, a*, and b* *(30)*, where L* = the total quantity of light reflected or brightness; a* = color ranging from red (positive values) to green (negative values); and b* = color ranging from blue (negative values) to yellow (positive values). Cutaneous sensitivity to UV radiation, or minimal erythema dose, was determined by measuring the minimal UV radiation dose able to provoke perceptible erythema of the skin 20–24 hours after exposure *(31)*. Minimal erythema dose was measured in an area of the body that is unexposed to the sun.

Sequencing of MC1R

MC1R was sequenced by two different laboratories at NCI-Frederick and the University of Pennsylvania in Philadelphia, using similar protocols. The 951-bp coding region of MC1R, either in its entirety or in smaller overlapping segments, was amplified by polymerase chain reaction (PCR) followed by complete

direct sequencing of the amplicon(s). Specific primer sequences and sequencing chemistries from the NCI laboratory are given below, and protocol details from the second laboratory *(32)* are available upon request.

The coding region of MC1R was amplified from genomic DNA extracted from patient blood samples using two sets of M13-tagged PCR primers: MC1R_1F (5′-GTA AAA CGA CGG CCA GTG AAG ACT TCT GGG CTC CCT C-3′) and MC1R_IIIR (5′-GGA AAC AGC TAT GAC CAT GGC GTG CTG AAG ACG ACA CT-3′); and MC1R_IVF (5′-GTA AAA CGA CGG CCA GTG TGC TGT ACG TCC ACA TGC T-3′) and MC1R_IVR (5′-GGA AAC AGC TAT GAC CAT GCT CTG CCC AGC ACA CTT AAA-3′). The underlined regions of the primers are specific to the target DNA. Amplification PCRs included 1× PCR buffer (Invitrogen High Fidelity PCR buffer); 1.5 m*M* $MgSO_4$; a 175 n*M* concentration of each pair of primers; a 50 n*M* concentration of each of the four dNTPs; and 1 unit of HiFi Platinum Taq polymerase (Invitrogen, Carlsbad, CA) and were carried out under the following conditions: 95 °C for 3 minutes, followed by 40 cycles of 95 °C for 1 minute, 58 °C for 1 minute, 72 °C for 1 minute, and finally an extension of 72 °C for 7 minutes. All PCR products were analyzed on a DNA 1000 labchip using "lab-on-a-chip" capillary electrophoresis (Agilent Technologies, Palo Alto, CA). All PCR products were treated with exonuclease I and shrimp alkaline phosphatase (USB Corporation, Cleveland, OH) as described *(26)*, and the PCR product was purified on Sephadex G-50 (Sigma-Aldrich, St. Louis, MO) as *(33)* described prior to sequencing. All PCR products were sequenced with the ABI prism BigDye Terminator Cycle Sequencing Kit 1.0 (Applied Biosystems, Foster City, CA) on an ABI3100 sequence analyzer using the sequence primer pairs 1F (5′- GCT CCC TCA ACT CCA CC-3′) and IR (5′- GAA GAC GAC ACT GGC CAC-3′) and M13F (5′-GTA AAA CGA CGG CCA GT-3′) and M13R (5′-GGA AAC AGC TAT GAC CAT G-3′). All sequences were analyzed and variants were detected using Mutation Surveyor (Soft Genetics LLC, State College, PA).

Genotyping of ASIP Polymorphism With 5′-Nucleotidase Assay

Sequences of the ASIP gene surrounding g.8818A>G (rs6058017) were submitted to an Applied Biosystems Assays-by-Design service. Primer sequences were 5′-CGTGCT CAGCCTCA-3′ and 5′-AGCCGCCCTGTTAG-3′, and probe sequences were 5′-VIC-CCGCGAGCGGGCAGGG-MGBNFQ-3′ and 5′-FAM-CCGCGAGCAGGCAGGGC-MGBNFQ-3′; VIC, MGBNFQ, and FAM are reporter dyes (Applied Biosystems). ASIP genotypes were determined using the 5′-nucleotidase assay as previously described *(34)*. For genotype sequence confirmation, 50 ng of DNA from samples known to be homozygous wild-type, heterozygous, or homozygous variant was selected from a panel of 88 anonymous, ethnically diverse DNAs (39% of European American origin, 34% of African American origin, and 27% of Hispanic origin) from a large AIDS cohort studied by the Laboratory of Genomic Diversity at the NCI. These samples were amplified using 2.5 units of AmpliTaq Gold enzyme (Applied Biosystems, Foster City, CA) in 4 m*M* $MgCl_2$, a 250 m*M* concentration of each nucleoside triphosphate, and 200 nmol of each primer, ASIP SEQF: 5′-GTAAAACGACGGCCAGTAGAAGG AGGCTTCGATG-3′ and ASIP SEQR: 5′-GGAAACAGCTATG ACCATGACCTTCGAGCAGCCTAT-3′, under the following cycling conditions: 95 °C for 10 minutes followed by 35 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds, 68 °C for 1 minute, followed by a final extension of 72 °C for 7 minutes. If necessary, the products were held overnight at 4 °C. PCR products were then treated with exonuclease I and shrimp alkaline phosphatase (USB Corporation) and were subjected to BigDye Terminator cycle sequencing in the presence of an M13 forward or reverse primer tag (M13 forward: 5′-GTAAAACGACGGCCAGT-3′ or M13 reverse: 5′-GGAAACAGCTATGACCATG-3′) for 15 minutes at 37 °C. PCR products were purified on Sephadex G-50, dried on a thermocycler (95 °C uncapped for 20 minutes), resuspended in 100% formamide, and electrophoresed on an ABI 3700 sequencer (Applied Biosystems, Foster City, CA). Forward and reverse trace files were examined visually, and sequences were determined using Mutation Explorer software (Soft Genetics, State College, PA).

Assessment of DNA Repair Capacity

DNA repair capacity was measured in cryopreserved lymphocytes of the subjects in the case–control study by the host–cell reactivation assay, as described by Athas et al. *(35)*. Briefly, plasmid DNA containing a chloramphenicol acetyltransferase (CAT) reporter gene was UV irradiated (254 nm at 0, 350, and 700 J/m^2) and then transfected into peripheral blood lymphocytes. The cells were incubated at 37 °C for 40 hours. After incubation, DNA repair capacity was calculated as the percentage of residual CAT gene expression after the repair of UV radiation–damaged plasmid DNA divided by that in undamaged plasmid DNA (100%). In all of the analyses, DNA repair capacity values measured after irradiation with UV doses of 350 and 700 J/m^2 were similar. Consequently, we report only results obtained after irradiation at 350 J/m^2.

CAT activity in unirradiated cells must be more than 1000 cpm to accurately measure DNA repair capacity with a signal-to-noise ratio that is at least double the background level (200 cpm) in cells with irradiated plasmids. Therefore, subjects with baseline CAT activities of less than 1000 cpm (51 of 183 melanoma patients, 27.9%, and 34 of 179 control subjects, 19.0%) were excluded from the analysis. We analyzed data from 132 melanoma patients and 145 control subjects from the case–control study. Details on the assay and related quality control measures are described by Landi et al. *(8)*.

Statistical Methods

Most MC1R variants were rare, and we were unable to independently assess the association between each MC1R variant and melanoma risk or pigmentation characteristics. Therefore, we analyzed three different risk categories, as follows (all compared with homozygous carries of the wild-type [WT] MC1R sequence): 1) carriers of any MC1R variant; 2) carriers of at least one RHC variant (RHC/RHC, RHC/NRHC, or RHC/WT) or of only non-RHC variants (NRHC/NRHC or NRHC/WT); and 3) carriers of two variants (RHC/RHC, RHC/NRHC or NRHC/NRHC). We also repeated the analyses excluding the RHC/NRHC genotypes from the RHC group to assess the association of "pure" red hair color variants with melanoma risk and pigmentation characteristics. Subjects were also characterized by the presence or absence of the ASIP polymorphism: SNP/WT or SNP/SNP vs. WT/WT.

We assessed the associations between pigmentation characteristics and genotypes in the control subjects of the case–control study using chi-square and Fisher's exact tests. In the family study we

used the generalized estimating-equations approach to account for correlations among controls from the same family. For most analyses, hair color was categorized into four groups: black, dark brown, light or reddish brown, and blond or red. Eye color was categorized into three groups: 1) black or dark brown; 2) light brown, brown-green, green or blue-green; and 3) light blue, blue, or grey. Skin color was categorized into three groups: dark/olive, medium, and light. Tanning ability after prolonged sun exposure was categorized into three groups: high, medium, and low. Freckling was categorized in two groups: presence and absence of freckles.

Odds ratios (ORs), 95% confidence intervals (CIs), and tests for trends were computed from unconditional logistic regression models (PROC GENMOD, SAS 8.2) for the case–control study and from conditional logistic regression models for the family study, conditioning on family (PROC PHREG, SAS 8.2). We adjusted the association for age, sex, presence of dysplastic nevi, tanning ability after prolonged sun exposure, and hair color by including these variables in the regression models. In the analyses involving only "pure" RHC and "pure" NRHC, we adjusted the models for age, sex, skin color, freckling, and Minolta b* index.

Odds ratios were computed for both studies combined by maximum likelihood estimation based on a likelihood function that multiplied conditional logistic regression terms for the families and unconditional logistic regression for the subjects from the case–control study, with adjustment for "study type," as well as for the variables mentioned above. For the family study, we also verified the validity of results from the conditional logistic models by comparing one randomly selected melanoma case patient from each family and all controls from the case–control study, using unconditional logistic regression. We obtained model-based 95% confidence intervals for the odds ratio estimates using the model information matrix. Computations were performed in Gauss (Aptech Systems Inc., Maple Valley, WA).

To tightly control for pigmentation characteristics, many variables would need to be included in the logistic regression models; however, many of these characteristics are highly correlated. To validate the pigmentation variables we ultimately included in the models, we compared the model containing each of these variables to the models that included all of the pigmentation characteristics in the form of independent factors from a factor analysis *(36)* (PROC FACTORS, SAS 8.2). Using both separate and combined data from the case–control and family studies, we constructed two factors for analysis based on the number of eigenvalues from the correlation matrix that were greater than 1 *(37)*. Factors were extracted using principal factor analysis and then rotated to derive factors that were orthogonal (i.e., independent). The statistically significant contributors to the first factor or "pigmentation" factor included hair color, eye color, skin color, and tanning ability. In addition, the Minolta b* skin color was a statistically significant contributor in the case–control study. The statistically significant contributors to the second factor or the "nevi" factor were number of nevi and presence of dysplastic nevi. For the case–control study, freckling was also a statistically significant contributor to the nevi factor. We then calculated factor scores for each individual and categorized the scores into equal-sized quartiles. This categorical variable was then used in logistic regression analysis that also controlled for age and gender. Models that included the original pigmentation and nevi variables were compared to models that used the derived factors using the likelihood ratio test. All P values are two-sided, where a P of <.05 was considered statistically significant.

Associations between categories of pigmentation characteristics and MC1R genotypes were assessed in logistic regression models to investigate effect modification as well as direct association with the genotypes. Poisson regression models were used when the number of nevi was the outcome. Results that suggested an interaction in stratified analyses were further examined for multiplicative interactions between MC1R variant alleles and the subjects' characteristic, e.g., pigmentation or ASIP polymorphism.

Analysis of MC1R could be performed on 165 case patients and 171 control subjects, and analysis of ASIP could be done on 166 case patients and 172 control subjects. Five control subjects and 13 case patients lacked both MC1R and ASIP data and were excluded from the analyses involving genotypes. DNA repair capacity was measured in 183 case patients and 179 control subjects. Different models may include different number of subjects because of missing values in some of the covariates.

Results

Frequency Distributions of MC1R and ASIP Variants

Overall, 32 different MC1R variants were found: 29 in subjects from the case–control study and 15 in subjects from the family study (Table 1). None of the nonsynonymous variants identified deviated from Hardy–Weinberg equilibrium. Three of these variants have not been reported previously, to our knowledge. One new variant, insA29, introduces a stop codon that results in a shorter, potentially nonfunctional protein. Frequencies of most variants were low. The most common variant was V60L. RHC variant alleles were more frequent in melanoma patients than in control subjects in both studies. Overall, 41 patients (26%) and 14 control subjects (8%) in the case–control study and 32 patients (38%) and 36 control subjects (18%) in the family study carried two variants of MC1R. In the case–control study, 41 melanoma patients (25%) and 46 control subjects (27%) carried the ASIP g.8818A>G polymorphism (data not shown).

Association of MC1R and ASIP Variants With Phenotypic Pigmentation and Nevi, by Sun Exposure and DNA Repair Capacity

The number of control subjects who carried either the RHC or NRHC variant alleles by age, gender, pigmentation characteristics, or nevi is shown in Table 2. In the case–control study, individuals who carried one or two RHC variants but not those who carried an NRHC variant had lighter hair (P_{trend} = .03) and lighter skin (P_{trend} = .005), were more likely to have freckles (P = .02) and dysplastic nevi (P = .02), and had more nevi (P<.001, in a Poisson regression model) than did those with wild-type alleles. In the family study, RHC variant genotypes were associated with tanning ability (P_{trend} = .02). All RHC carriers (16/16) had freckles. By contrast with MC1R, the ASIP polymorphism was not associated with pigmentation characteristics (data not shown). It was also not associated with MC1R variants categorized by RHC or NRHC (OR = 0.5; 95% CI = 0.2 to 1.7, and OR = 1.2; 95% CI= 0.6 to 2.6, for RHC and NRHC, respectively).

The association between carrying RHC variant alleles and having freckles was modified by DNA repair capacity. In subjects with DNA repair capacity below the median value, the odds ratio was 5.0 (95% CI = 1.4 to 17.9), whereas in subjects with

Table 1. Subjects with single and multiple MC1R variant alleles identified from a case–control study and family study of melanoma from northeastern Italy*

MC1R variant	Nucleotide change	Case–control study				Family study				No. of families‡
		Melanoma patients (N = 165)		Control subjects (N = 171)		Melanoma patients (N = 84)		Control subjects (N = 203)		
		Single	Mult.†	Single	Mult.†	Single	Mult.†	Single	Mult.†	
insA29§	insA29	—	—	—	—	3 (3.6)	—	2 (1.0)	—	2
T19I	56C>T	1 (0.6)	—	—	—	—	—	—	—	—
insA86	insA86	—	—	1 (0.6)	—	—	—	—	—	—
C35Y	104G>A	1 (0.6)	—	—	—	—	—	—	—	—
F45L	133T>C	—	—	2 (1.7)	—	2 (2.4)	—	3 (1.5)	—	3
V60L	178G>T	50 (30.3)	5 (3.0)	44 (25.7)	5 (2.9)	26 (30.9)	2 (2.4)	59 (29.1)	11 (5.4)	34
V60G§	179T>G	1 (0.6)	—	—	—	—	—	—	—	—
delG200	delG200	2 (1.2)	—	—	—	—	—	—	—	—
D84E	252C>A	1 (0.6)	—	—	—	8 (9.5)	—	15 (7.4)	—	8
V92M	274G>A	12 (7.3)	—	16 (9.4)	1 (0.6)	14 (16.7)	1 (1.2)	29 (14.3)	1 (0.5)	14
T95M	284C>T	1 (0.6)	—	—	—	1 (1.2)	—	—	—	1
G104S	310G>A	1 (0.6)	—	—	—	—	—	—	—	—
V112M	334G>A	1 (0.6)	—	—	—	—	—	—	—	—
R142H	425G>A	6 (3.6)	—	2 (1.2)	—	3 (3.6)	—	2 (1.0)	—	3
R151C	451C>T	39 (23.6)	2 (1.2)	16 (9.4)	—	6 (7.1)	1 (1.2)	14 (6.9)	—	12
Y152STP	456C>A	1 (0.6)	—	—	—	—	—	—	—	—
I155T	464T>C	3 (1.8)	—	—	—	1 (1.2)	—	—	—	1
R160W	478C>T	9 (5.5)	—	9 (5.3)	—	3 (3.6)	—	5 (2.5)	—	5
R163Q	488G>A	10 (6.1)	—	4 (2.3)	—	10 (11.9)	—	10 (4.9)	1 (0.5)	14
A171D	512C>A	2 (1.2)	—	1 (0.6)	—	—	—	—	—	—
insC537	insC537	1 (0.6)	—	—	—	—	—	—	—	—
D184H	550G>C	1 (0.6)	—	—	—	—	—	—	—	—
R213W	637C>T	1 (0.6)	—	—	—	3 (3.6)	—	2 (1.0)	—	3
A218T	652G>A	1 (0.6)	—	—	—	—	—	—	—	—
R223Q	668G>A	1 (0.6)	—	—	—	—	—	—	—	—
aa227§	681G>A	—	—	—	—	—	—	1 (0.5)	—	1
P230L	689C>T	1 (0.6)	—	—	—	—	—	—	—	—
G248V	743G>T	—	—	1 (0.6)	—	—	—	—	—	—
P256S	766C>T	2 (1.2)	—	—	—	—	—	—	—	—
K278E	832A>G	—	—	—	—	2 (2.4)	—	1 (0.5)	—	1
D294H	880G>C	11 (6.7)	—	6 (3.5)	—	7 (8.3)	—	9 (4.4)	—	9
T308M	923C>T	—	—	1 (0.6)	—	—	—	—	—	—

*MC1R = melanocortin-1 receptor. Empty cells indicate absence of MC1R variants (WT MC1R).

†Mult. = subjects who were homozygous for a given variant MC1R allele.

‡Number of families with family members who carry one or more of a given MC1R variant allele.

§MC1R variant alleles that have not been reported before.

DNA repair capacity above the median value, the odds ratio was 1.6 (95% CI = 0.4 to 6.8). The association between RHC variants and having freckles was also modified by sun exposure. In subjects with high numbers of hours of recreational sun exposure (>median of 861 hours), the odds ratio was 5.6 (95% CI = 1.3 to 23.5), whereas in subjects with low sun exposure, the odds ratio was 2.3 (95% CI = 0.5 to 10.5). Among 13 individuals with RHC alleles and high sun exposure, those with low repair capacity (n = 8) had an unadjusted odds ratio of having freckles of 26.3 (95% CI = 2.5 to 280.2), whereas those with high repair capacity and high sun exposure had an unadjusted odds ratio of having freckles of 1.4 (95% CI = 0.2 to 9.9). By contrast with the RHC and freckle association, the association between RHC and number of nevi was not substantially modified by sun exposure or DNA repair capacity (data not shown). However, in the subgroup of subjects with high sun exposure and RHC (n = 53), the association between RHC and nevi was stronger in those with low DNA repair capacity (n = 27, OR = 2.4; 95% CI = 1.5 to 3.8) than in those with high DNA repair capacity (OR = 1.0; 95% CI = 0.8 to 1.2). Thus, these data suggest that freckles and nevi may increase in subjects with intense sun exposure and low capacity to repair the UV-induced DNA damage. However, these findings were based on very small numbers and require confirmation.

Association of MC1R and ASIP With Instrument-Based Measures of Pigmentation and UV Radiation Sensitivity

Overall, there was no statistically significant association between the MC1R variants or the ASIP polymorphism with the Minolta L* and a* skin colors. Similarly, there was no statistically significant association between variants in either gene with UV radiation sensitivity, as measured by minimum erythema dose. However, we found a strong association between RHC and NRHC MC1R variants and high Minolta b* (i.e., yellowish) color of the skin ($P = .006$). When we stratified the analysis by sex, the association of MC1R variants with Minolta b* was statistically significant in men ($P = .007$) but not in women ($P > .44$). This difference may stem from men's generally thicker epidermis *(38)*.

Association of MC1R and ASIP Variants With Melanoma Risk

The association between MC1R variants and melanoma risk in the case–control and the family studies separately as well as in both studies combined is shown in Table 3. In all three sets of analyses, subjects carrying any variant, one or more NRHC variants, one or

Table 2. Number of control subjects with pure RHC and NRHC MC1R variants in a case–control study and a family study of melanoma from northeastern Italy by subject characteristic*

	Case–control study			Family study		
	WT	RHC	NRHC	WT	RHC	NRHC
Characteristic	N	N (%)†	N (%)‡	N	N (%)†	N (%)‡
Age at interview, y						
<35	16	10 (38.5)	13 (44.8)	25	5 (16.7)	53 (68.0)
35–44	17	5 (22.7)	19 (52.8)	12	1 (7.7)	16 (57.1)
45–54	18	6 (25.0)	16 (47.1)	12	3 (20.0)	20 (62.5)
55+	20	5 (20.0)	21 (51.2)	10	8 (44.4)	23 (69.7)
Sex						
Male	38	13 (28.3)	30 (44.1)	28	9 (24.3)	50 (64.1)
Female	33	13 (25.5)	39 (54.2)	33	9 (21.3)	64 (66.0)
Eye color						
Dark	27	12 (30.8)	27 (50.0)	22	9 (29.0)	60 (73.2)
Medium	37	14 (27.5)	31 (45.6)	17	4 (19.1)	29 (63.0)
Light	6	0 (0.0)	11 (64.7)	20	4 (16.7)	22 (52.4)
Skin color						
Dark	17	1 (5.6)	10 (37.0)	10	0 (0.0)	12 (54.6)
Medium	37	12 (24.5)	36 (49.3)	34	11 (24.4)	75 (68.8)
Light	17	13 (43.3)§	23 (57.5)	15	6 (28.57)	25 (62.5)
Natural hair color						
Black	14	1 (6.7)	7 (33.3)	3	2 (40.0)	5 (62.5)
Dark brown	42	16 (27.6)	37 (46.8)	32	9 (22.0)	81 (71.7)
Light brown/reddish brown	12	5 (29.4)	22 (64.7)	21	4 (16.0)	23 (52.3)
Blond/red	3	4 (57.1)§	3 (50.0)	3	2 (40.0)	3 (50.0)
Freckling						
No	45	10 (18.2)	40 (47.1)	7	0 (0.0)	12 (63.2)
Yes	25	16 (39.0)§	28 (52.8)	49	16 (24.6)	98 (66.7)
Dysplastic nevi						
No	54	13 (19.4)	51 (48.6)	23	4 (14.8)	54 (70.13)
Indeterminate	7	5 (41.6)	10 (58.8)	5	4 (44.4)	13 (72.2)
Yes	10	8 (44.4)§	8 (44.4)	31	9 (22.5)	45 (59.2)
No. of nevi						
0–15	19	3 (13.6)	18 (48.7)	23	5 (17.9)	43 (65.2)
16–25	10	3 (23.1)	12 (54.6)	10	1 (9.1)	11 (52.4)
26–46	12	8 (40.0)	19 (61.3)	10	2 (16.7)	25 (71.4)
47+	18	7 (28.0)‖	13 (41.9)	12	5 (29.4)	20 (62.5)
Tanning ability						
Deep tan	28	7 (20.0)	23 (45.1)	17	2 (10.5)	33 (66.0)
Moderate tan	34	13 (27.7)	34 (50.0)	27	6 (18.2)	51 (65.4)
Mild or no tan	7	5 (41.7)	11 (61.1)	10	6 (37.5)‡	17 (63.0)

*RHC = red hair color variant (RHC/WT or RHC/RHC genotypes); NRHC = non-red hair color variant (NRHC/WT or NRHC/NHRC genotypes); WT = wild-type (WT/WT); MC1R = melanocortin-1 receptor.

†Percentages obtained by dividing the number of RHC variants by the sum of WT and RHC variants.

‡Percentages obtained by dividing the number of NRHC variants by the sum of WT and NRHC variants in each cell.

§Statistically significant associations ($P = .04$, skin color; $P = .03$, hair color; $P = .04$, tanning ability, test for trend; $P = .02$, freckling; $P = .02$, dysplastic nevi, Fisher's exact test).

‖The association of RHC with number of nevi was significant in a Poisson regression model ($P<.001$).

more RHC variants, "pure" RHC variants, or two of any MC1R variants had a two- to threefold increase in melanoma risk compared with subjects who carried the wild-type sequence, after adjustment for age, sex, hair color, tanning ability, and presence of dysplastic nevi. These findings were confirmed in models that adjusted for skin color, freckling, or "pigmentation" and "nevi" factors (data not shown). For the family study, we verified the validity of our results by comparing one randomly selected melanoma patient from each family and all control subjects from the case–control study. Although this analysis was based on a smaller number of subjects, the results were similar (data not shown).

Association of MC1R Variants With Melanoma Risk in Families Carrying CDKN2A Mutations

Of 55 families, only four were positive for CDKN2A mutations. Family members of two of the four families with CDKN2A mutations had no MC1R variant alleles. The exclusion of these four families did not substantially change the association between MC1R and melanoma risk (data not shown). We also found no association between MC1R variants and presence of multiple melanomas (12 cases) or age at first melanoma diagnosis (data not shown).

Carrying the ASIP polymorphism was not associated with melanoma risk overall (OR = 1.1; 95% CI = 0.6 to 1.8). However, we did find some associations with the ASIP SNP in selected subgroups (based on small numbers), as described below.

Association Between MC1R Variants and Melanoma Thickness or Body Site

We investigated the association between melanoma thickness, an indicator of melanoma progression, and MC1R variants in case patients from the case–control study. Case patients with any MC1R variant were at three- to fourfold risk of having thick melanoma

Table 3. Association between melanocortin-1 receptor (MC1R) variants and melanoma risk in a case–control study, a family study, and combined studies of melanoma from northeastern Italy*

	Case–control study†			Family study‡			Combined studies§		
MC1R status	Cases (n = 162)	Controls (n = 167)	OR (95% CI)	Cases (n = 70)	Controls (n = 178)	OR (95% CI)	Cases (n = 228)	Controls (n = 345)	OR (95% CI)
No variant	35	69	1.0 (referent)	13	54	1.0 (referent)	47	123	1.0 (referent)
Any variant	127	98	2.12 (1.2 to 3.6)	57	124	2.98 (1.0 to 8.6)	181	222	2.12 (1.3 to 3.4)
NRHC variants‖	56	68	2.04 (1.1 to 3.6)	43	101	2.85 (1.0 to 8.4)	98	169	2.10 (1.3 to 3.4)
≥1 RHC variants¶	71	30	2.38 (1.2 to 4.7)	14	23	3.47 (0.9 to 13.9)	83	53	1.86 (1.1 to 3.1)
Two variants in the same subject#	41	14	3.37 (1.5 to 7.4)	30	30	2.98 (1.4 to 6.2)	71	44	3.91 (3.3 to 4.6)

*All analyses adjusted for age, sex, presence of dysplastic nevi, tanning ability (fitted with trend) and hair color (fitted with trend). OR = odds ratios; CI = confidence interval; NRHC = non-red hair color; RHC = red hair color.

†Unconditional regression analysis.

‡Conditional regression analysis (conditioning on family). Excluding four families (nine subjects) with CDKN2A mutation carriers did not substantially modify the results.

§Maximum likelihood estimation.

‖Only non-red hair color variants: NRHC/WT (wild-type) or NRHC/NRHC.

¶At least one RHC variant: RHC/WT or RHC/NRHC or RHC/RHC. The exclusion of the subjects with RHC/NRHC genotypes from the RHC group did not substantially change the results.

#RHC/RHC or NRHC/NRHC or RHC/NRHC.

lesions compared to melanoma patients who were homozygous wild type for MC1R, after controlling for age, sex, hair color, tanning ability, and dysplastic nevi (Table 4). The addition of DNA repair capacity to the model did not substantially change the results (data not shown). We also investigated the association between presence of MC1R variants and the physical location of the melanomalesions. In the case–control study, carrying multiple MC1R variants was statistically significantly associated with melanoma in areas of the body that are continuously exposed to the sun (e.g., OR of having a melanoma on face, neck, or back of hands = 8.5; 95% CI = 2.2 to 33.6), although this conclusion was based on small numbers. In contrast, carrying the ASIP polymorphism was inversely associated with melanoma at those same body sites (OR = 0.08; 95% CI = 0.01 to 0.9). There was no association between carrying MC1R variants and developing melanoma in areas of the body that are intermittently or rarely exposed to sun (data not shown). We were unable to confirm these results in the family study because so few subjects had melanomas in continuously sun-exposed areas of the body. However, there was a statistically significant inverse association between carrying any MC1R variant (OR = 0.01; 95% CI = 0.001 to 0.3) or multiple MC1R variants (OR = 0.05; 95% CI = 0.005 to 0.4) and melanoma in areas of the body that are rarely, if ever, exposed to the sun (e.g., soles of the feet, nail beds, and mucosa).

Association of MC1R Variants With Melanoma Risk by Subjects' Characteristics

We explored potential effect modifications of the association between MC1R genotypes and melanoma risk (Table 5) by nevi- and pigmentation-related variables. Although the results are based on small numbers, the association between RHC variants and melanoma was stronger in subjects who had characteristics that are associated with being at lower risk of developing melanoma, (i.e., few nevi, no dysplastic nevi, no freckling, low sun exposure, dark hair, and low Minolta b* index). In particular, the association between RHC variants and melanoma was modified by freckling, and this effect modification was statistically significant in a test for multiplicative interaction ($P = .02$). In subjects with NRHC, melanoma risk was not statistically significantly modified by nevi- and pigmentation-related variables. We could not explore this association in the family study because of the low frequency of subjects with pure RHC genotype, even after inclusion of the D84E variant, as suggested by Duffy et al. *(18)*.

In the case–control study, we also investigated whether DNA repair capacity or ASIP genotype modified the association between RHC or NRHC MC1R variants and melanoma risk. Neither DNA repair capacity nor the ASIP polymorphism modified the association, with one exception: subjects with one or two RHC variant alleles and the ASIP polymorphism had a much higher risk of melanoma (OR = 26.7; 95% CI = 2.5 to 289.8, $P = .02$, test for multiplicative interaction) than did subjects who were homozygous for the wild-type allele of MC1R and did not carry the ASIP polymorphism. However, this finding should be taken with caution, given the small number of subjects in this subgroup analysis.

Discussion

UV radiation is a well-established risk factor for melanoma, with a range of effects on the skin. One effect of UV radiation is

Table 4. Association between melanocortin-1 receptor (MC1R) variants and melanoma thickness in a case–control study of melanoma from northeastern Italy*

MC1R status	< Median thickness (n = 74)	> Median thickness (n = 73)	OR‡ (95% CI)
No variant	21	10	1.0 (referent)
Any variant	53	63	2.92 (1.20 to 7.10)
NRHC variants†	30	34	3.24 (1.19 to 8.83)
≥1 RHC variants‡	23	29	2.70 (1.04 to 7.01)
Two variants in the same subject§	15	23	4.05 (1.32 to 12.40)

*Median of melanoma thickness was 1.06 mm. Models were adjusted for age, sex, presence of dysplastic nevi, tanning ability (fitted for trend), and hair color (fitted for trend). OR = odds ratio; CI = confidence interval; NRHC = non-red hair color; RHC = red hair color.

†Only NRHC variants: NRHC/WT (wild-type) or NRHC/NRHC.

‡At least 1 RHC variant: RHC/WT or RHC/NRHC or RHC/RHC.

§RHC/RHC or NRHC/NRHC or RHC/NRHC.

Table 5. Melanocortin-1 receptor (MC1R) variants and melanoma risk by subjects' characteristics in a case–control study of melanoma in Italy*

Characteristics	RHC			NRHC		
	Cases†	Controls†	OR‡ (95% CI)	Cases†	Controls†	OR§ (95% CI)
Nevi, median						
Below	10/22	9/53	3.57 (1.1 to 11.7)	27/39	39/84	2.24 (1.0 to 5.2)
Above	17/35	14/37	1.05 (0.3 to 3.3)	39/57	27/50	1.80 (0.8 to 4.1)
DN						
No	13/28	13/66	2.24 (0.8 to 6.5)	33/48	51/105	2.15 (1.0 to 4.5)
Yes	13/28	8/18	0.88 (0.2 to 3.4)	25/40	8/18	1.80 (0.5 to 6.1)
Freckling						
No	16/29	10/55	6.20 (1.9 to 20.1)	36/49	40/85	3.10 (1.4 to 6.8)
Yes	15/37	16/41	0.80 (0.3 to 2.6)	38/60	28/53	1.66 (0.7 to 3.7)
Nevi factor,‖ median						
Below	10/22	8/54	4.67 (1.3 to 17.4)	26/38	41/87	2.06 (0.9 to 4.9)
Above	15/33	14/33	1.02 (0.4 to 2.9)	35/53	23/42	1.60 (0.7 to 3.8)
Sun exposure, median						
Below	19/36	10/37	3.09 (1.0 to 9.2)	37/53	33/61	1.89 (0.8 to 4.2)
Above	11/27	13/53	1.08 (0.3 to 3.7)	35/51	32/72	2.91 (1.3 to 6.4)
DNA repair capacity, median						
Below	17/38	15/54	1.47 (0.5 to 4.0)	45/66	43/83	2.07 (1.0 to 4.1)
Above	14/28	11/42	1.84 (0.6 to 6.1)	30/44	26/57	2.46 (1.1 to 5.7)
Hair color						
Dark	19/39	17/72	2.88 (1.1 to 7.3)	47/67	44/100	2.88 (1.5 to 5.7)
Light	12/27	9/24	1.18 (0.3 to 4.2)	26/41	25/40	1.06 (0.4 to 2.7)
Minolta b*, median						
Below	19/35	12/42	3.32 (1.0 to 10.8)	45/61	36/67	2.37 (1.1 to 5.0)
Above	12/31	14/54	1.60 (0.5 to 4.7)	30/41	33/73	2.17 (1.0 to 4.7)
ASIP						
No	15/42	21/73	1.14 (0.5 to 2.28)	57/84	47/99	2.47 (1.3 to 4.7)
Yes	16/24	4/22	26.7 (2.5 to 289.8)	16/24	21/40	2.10 (0.7 to 6.6)
Pigmentation factor,¶ median						
Below	11/24	13/58	2.42 (0.7 to 7.9)	27/40	35/80	2.05 (0.9 to 4.9)
Above	14/31	9/29	1.48 (0.5 to 4.7)	34/51	29/49	1.46 (0.6 to 3.5)

*RHC = red hair color; NRHC = non-red hair color; OR = odds ratio; 95% CI = 95% confidence interval; DN = dysplastic nevi; ASIP = Agouti Signaling Protein.
†Number of MC1R variant carriers/total number of subjects in each stratum.
‡Models adjusted by age, sex, skin color, freckling, and DN. Adding sun exposure in the model did not substantially modify the results.
§Models adjusted by age, sex and Minolta b* index (tendency to yellowish color). For the model stratified by freckling, DN was also added.
‖Statistically significant contributors were number of nevi, presence of dysplastic nevi and freckling. Model was adjusted for age, sex, and "pigmentation" factor.
¶Significant contributors: hair color, eye color, tanning ability, Minolta b*. Model was adjusted for age, sex, and "nevi" factor.

stimulation of pigment production, possibly through the MC1R gene. We examined the association of variants in MC1R, as well as a particular variant of the ASIP gene, in both sporadic and familial melanoma in a Mediterranean population. MC1R was highly polymorphic in our study subjects, which is consistent with what has been reported in populations with skin generally fairer than in this Mediterranean population. The frequency of RHC variant alleles, particularly in the family study, was lower than stated by previous reports in fairer-skinned populations, while NRHC variant alleles, particularly V60L, were higher *(18)*.

We observed a two- to fourfold-increased risk of melanoma among individuals who carried MC1R variant alleles, similar to the increased risks reported in analyses of MC1R and melanoma risk in subjects of Celtic or Germanic origin *(17,19)*, but we found no association between melanoma risk and the ASIP polymorphism. Furthermore, the magnitude of the odds ratios was similar in the case–control and family studies, suggesting a similar role of the gene in sporadic and familial melanoma in this population. This similarity may be related to the lack of germline CDKN2A mutations in most families in the family study. In fact, only two families had members who carried a germline CDKN2A mutation and also carried an MC1R variant allele. Interestingly, sporadic melanoma patients had more variant MC1R alleles, including RHC alleles, than did familial melanoma patients. This difference may be related to the smaller number of independent subjects in the family study. Alternatively, genes other than MC1R or CDKN2A may have a greater role in familial melanoma in this population. Larger family studies from Mediterranean populations will be required to further investigate these associations.

Individuals carrying MC1R variants were at increased risk of melanoma even after controlling for hair color and tanning ability or for a "pigmentation" factor that took into account the combined effect of hair color, tanning ability, skin color, eye color, and Minolta b* index. The association between MC1R variants and melanoma also remained statistically significant after adjustment for the presence of dysplastic nevi or for a "nevi" factor that took into account number of nevi, presence or absence of dysplastic nevi, and freckles. This suggests that MC1R variants increase the risk for melanoma beyond the risk due to fair pigmentation, as previously seen in other populations *(17,39,40)*, but also beyond the risk associated with nevi. However, the association between MC1R and melanoma was modified by these known phenotypic factors (i.e., pigmentation, freckling, and nevi). In fact, the association between RHC variants and melanoma risk was stronger in subjects without dysplastic nevi or with few nevi, no freckles, or darker pigmentation, suggesting that carrying RHC variant alleles is an independent risk factor for melanoma among phenotypically low-risk individuals. This finding is consistent with the associations reported in individuals with darker skin *(19)* and high tanning ability *(41)* in

Australian and English populations, respectively, but contrasts with the associations found in a small study of French subjects *(40)*.

A number of possible hypotheses could explain the stronger association between MC1R variants and melanoma risk in subjects with fewer risk factors. One explanation may be that MC1R has an additional role that is unrelated to its function in regulating pigmentation *(11)*. Indeed, α-MSH influences growth and development of melanocytes and melanoma cells in vitro *(42)* through MC1R. MC1R variants could alter the effect of α-MSH on melanoma cell behavior independent of their effects on pigmentation. Second, nevi represent melanocyte cell clones that have undergone cellular expansion followed by growth arrest *(43)*. MC1R may play a role in enhancing melanocyte activity and at the same time limiting clonal expansion of melanocytes *(18)*. If MC1R variants alter the latter function, clones may escape growth arrest, and individuals may develop melanoma; if melanocytic clones do not escape growth arrest, individuals may develop nevi or, in the absence of melanocyte clones, freckles. These changes may be affected by other factors. In our study, intense sun exposure, coupled with low DNA repair capacity, appeared to increase the association of RHC alleles of MC1R with nevi and freckling. Once melanoma develops, DNA repair (or at least the DNA repair that is measured in lymphocytes) may no longer be relevant, as suggested by the lack of an impact by DNA repair capacity on the association between MC1R variants and melanoma risk or progression. Third, nonfunctional MC1R may alter glucocorticoid induction mediated by ACTH. Glucocorticoid use (either topical or systemic) has been associated with decreased melanoma risk in a previous study in this population *(44)*. One might speculate that altered MC1R function may decrease the potentially protective effect of ACTH-induced glucocorticoids. Finally, it is possible that the different MC1R variants have unique roles relative to pigmentation or nevi/melanoma formation. In fact, both RHC and NRHC alleles were associated with melanoma risk, but only RHC alleles were associated with nevi or many pigmentation characteristics. This finding is especially important since carriers of NRHC alleles may erroneously consider themselves to be at lower risk for melanoma, especially if they have darker skin or no nevi.

We also found that melanoma patients with multiple MC1R variants had more melanomas in continuously sun-exposed areas of the body (e.g., face, neck, back of hands) than did individuals who had the consensus sequence, whereas the ASIP polymorphism was inversely associated with melanoma at these body sites. These findings need to be reproduced, given the small number of subjects with melanomas at these body sites. UV radiation is known to induce the release of α-MSH and to increase the expression of MC1R in the epidermis *(45)*. Nonfunctional MC1R may alter local responses to UV radiation *(46,47)*. In fact, it has been shown that melanocytes with nonfunctional MC1R are more sensitive to the cytotoxic effect of UV radiation than are melanocytes expressing functional MC1R in vitro *(48)*.

MC1R variants were statistically significantly associated with melanoma thickness, which is an indicator of disease progression. This intriguing association may reflect a delay in diagnosis or a more aggressive form of melanoma in subjects with MC1R variants, and it may be related to the inability of nonfunctional MC1R variants to reduce growth and progression of melanoma cells *(49)*. In addition, nonfunctional MC1R may reduce the glucocorticoid-related immunosuppressive effect that follows binding of ACTH with MC1R. Individuals with red hair, who are more likely to carry RHC variants, might be expected to have thicker lesions. We are not aware of any reports on the association between red hair and melanoma thickness. However, a study reported an interaction among cytochrome p450 2D6, MC1R and pigmentation with melanoma thickness *(50)*. Further study of melanoma progression is thus required, given the importance of identifying subjects who would benefit from targeted therapies. We did not examine the association with melanoma thickness in the family study because frequent screenings may bias melanoma thickness in younger generations. Indeed, melanoma lesions in the patients from the case–control study (median thickness = 1.06 mm) were thicker than those from melanoma patients from the family study (median thickness = 0.60 mm).

The current study has several limitations. One is the low statistical power in some subgroup analyses, which showed imprecise odds ratio estimates with wide confidence intervals. In addition, it was not possible to examine the association of each individual MC1R variant with melanoma risk or pigmentation. Finally, it is important to note the possibility of false-positive findings due to multiple analyses. Further confirmatory work is thus needed, particularly for the results based on small numbers of subjects.

In conclusion, carrying MC1R variants, particularly RHC, was associated with fair pigmentation, more nevi, and increased melanoma risk in this Italian population. In contrast, the g.8818A>G ASIP polymorphism was not associated with pigmentation or melanoma risk overall, even though it may interact with RHC variants in modifying melanoma risk. Assessment of the role of pigmentation genes in melanoma risk may identify high-risk subjects who could benefit from prevention strategies and targeted therapies.

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Notes

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